SortMeRNA User Manual

Evguenia Kopylova
evguenia.kopylova@lifl.fr

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Contents

1 Introduction

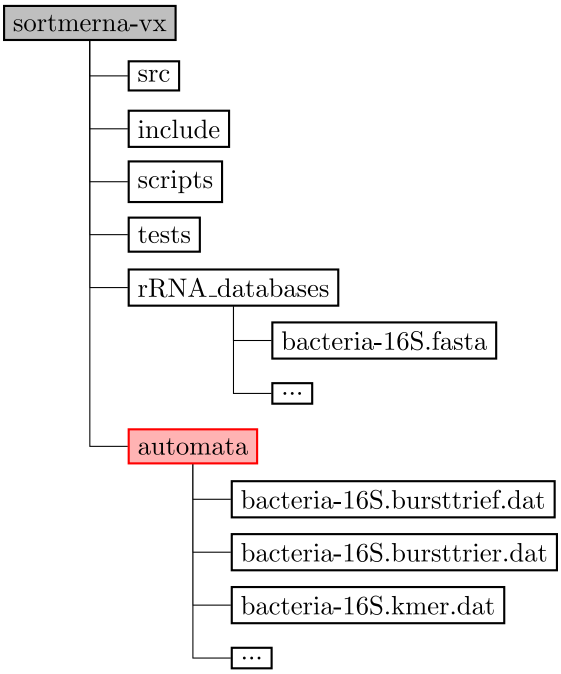
SortMeRNA is a software designed to filter metatranscriptomic reads data. It takes as input a file of reads (fasta or fastq format) and an rRNA database file, and sorts apart the accepted reads and the rejected reads into two files specified by the user.

For questions & help, please contact:

```
1. Evguenia Kopylova     evguenia.kopylova@lifl.fr
2. Laurent Noe           laurent.noe@lifl.fr
3. Helene Touzet         helene.touzet@lifl.fr
```

2 Installation

Figure 1: `sortmerna-vx` directory tree



2.1 Install from source code

1. Download `sortmerna-vx.tar.gz` from `http://bioinfo.lifl.fr/RNA/sortmerna`
2. Extract the source code package into a directory of your choice, for quick installation without tests, type:

Quick Installation (no testing)

```
> ./configure
> make
> make install (with root permissions)

Go to Step 5.
```

3. For installation with testing, proceed by typing:

   ```
   > ./configure
   > make
   > make check
   ```

4. At this point, two executables `buildtrie` and `sortmerna` will be located in the `sortmerna-vx` directory, as well as the indexed rRNA databases in `sortmerna-vx/automata`. If the user would like to install the executables into `/usr/local/bin` and the `sortmerna-vx` directory into `/usr/local/sortmerna-vx`, then type,

   ```
   > make install (with root permissions)
   > make installcheck
   ```

5. SortMeRNA indexes a database by writing its contents to the '`/automata`' folder (see Figure 1), which is initially located in the directory '`/sortmerna-vx/automata`'. It is essential to set the `$SORTMERNADIR` environmental variable to the path where the '`/automata`' folder is found, so that the program may read and write from it. The user may move the '`/automata`' folder to a workspace with larger memory, separately from the directory '`/sortmerna-vx`'. To find the path of the '`/automata`' folder, go into the directory where it is located (we assume here it is located under the directory `sortmerna-vx`) and type,

   ```
   > pwd
   /path/to/sortmerna-vx
   ```

   Open the ~/.bashrc (or ~/.profile) file in any editor and add the line,

   ```
   > export SORTMERNADIR="/path/to/sortmerna-vx"
   ```

   If the user installed SortMeRNA in Step (2) or Step (4), go to Step (6). Otherwise, the user must also include the path of the executable files in the `PATH` variable (we assume `buildtrie` and `sortmerna` are located in the `sortmerna-vx` directory),

   ```
   > export PATH="$PATH:/path/to/sortmerna-vx"
   ```

6. Run the ~/.bashrc (or ~/.profile) file to add the variable `$SORTMERNADIR` and update the variable `$PATH` in the list of environment variables,

   ```
   > source ~/.bashrc
   or
   ```

```
> source ~/.profile
```

7. Check the path of the executables (if installed manually),

```
> which sortmerna buildtrie
/path/to/sortmerna-vx/sortmerna
/path/to/sortmerna-vx/buildtrie
```

8. Check that $SORTMERNADIR has been added,

```
> echo $SORTMERNADIR
/path/to/sortmerna-vx  (if it has not been added,
                        this path will be empty)
```

9. To begin using SortMeRNA, type 'buildtrie -h' or 'sortmerna -h'. If the user installed SortMeRNA in Step 3, then the public rRNA databases distributed with SortMeRNA have been indexed in $SORTMERNADIR/automata and the user may directly run the command sortmerna. Otherwise, the user must firstly index the databases with the command buildtrie before they can run the command sortmerna.

2.2 Install from precompiled code

1. Download the latest binary distribution of SortMeRNA from http://bioinfo.lifl.fr/RNA/sortmerna
2. Extract the source code package into a directory of your choice,

```
> tar -zxvf sortmerna-vx.tar.gz
> cd sortmerna-vx
```

3. SortMeRNA indexes a database by writing its contents to the '/automata' folder (see Figure 1), which is initially located in the directory '/sortmerna-vx/automata'. It is essential to set the $SORTMERNADIR environmental variable to the path where the '/automata' folder is found, so that the program may read and write from it. The user may move the '/automata' folder to a workspace with larger memory, separately from the directory '/sortmerna-vx'. To find the path of the '/automata' folder, go into the directory where it is located (we assume here it is located under the directory sortmerna-vx) and type,

```
> pwd
/path/to/sortmerna-vx
```

4. Open the ~/.bashrc (or ~/.profile) file in any editor and add the line,

```
> export SORTMERNADIR="/path/to/sortmerna-vx"
> export PATH="$PATH:/path/to/sortmerna-vx"
```

5. Run the ~/.bashrc (or ~/.profile) file to add the variable $SORTMERNADIR and update the variable $PATH in the list of environment variables,

```
> source ~/.bashrc
or
> source ~/.profile
```

6. Check that $SORTMERNADIR has been added,

   ```
   > echo $SORTMERNADIR
   /path/to/sortmerna-vx   (if it has not been added,
                            this path will be empty)
   ```

7. Check that the $PATH has been updated with the additional directory search path,

   ```
   > echo $PATH
   /usr/local/bin:/usr/bin:...:/path/to/sortmerna-vx
   ```

8. Check the path of the executables,

   ```
   > which sortmerna buildtrie
   /path/to/sortmerna-vx/sortmerna
   /path/to/sortmerna-vx/buildtrie
   ```

9. To begin using SortMeRNA, type '`buildtrie -h`' or '`sortmerna -h`'. If the user installed SortMeRNA in Step 3, then the public rRNA databases distributed with SortMeRNA have been indexed in `$SORTMERNADIR/automata` and the user may directly run the command `sortmerna`. Otherwise, the user must firstly index the databases with the command `buildtrie` before they can run the command `sortmerna`.

2.3 Uninstall

If the user installed SortMeRNA using the command '`make install`', then they can use the command '`make uninstall`' to uninstall SortMeRNA (with root permissions).

3 Databases

SortMeRNA comes prepackaged with 8 databases,

representative database	**id %**	**average id %**	**# seq**	**origin**	**# seq**	**filtered to remove**
silva-bac-16s-database-id85.fasta	85	91.6	8174	SILVA SSU Ref NR v.111	244077	23s
silva-arc-16s-database-id95.fasta	95	96.7	3845	SILVA SSU Ref NR v.111	10919	23s
silva-euk-18s-database-id95.fasta	95	96.7	4512	SILVA SSU Ref NR v.111	31862	26s,28s,23s
silva-bac-23s-database-id95.fasta	98	99.4	3055	SILVA LSU Ref v.111	19580	16s,26s,28s
silva-arc-23s-database-id95.fasta	98	99.5	164	SILVA LSU Ref v.111	405	16s,26s,28s
silva-euk-28s-database-id95.fasta	98	99.1	4578	SILVA LSU Ref v.111	9321	18s
rfam-5s-database-id98.fasta	98	99.2	59513	RFAM	116760	–
rfam-5.8s-database-id98.fasta	98	98.9	13034	RFAM	225185	–

The tool UCLUST was used to reduce the size of the original databases.

id %: members of the cluster must have identity at least this % id with the representative sequence
average id %: average identity of a cluster member to the representative sequence

Remark: The user must first index the fasta database by using the command `buildtrie` and then filter reads against the database using the command `sortmerna`.

4 How to run SortMeRNA

4.1 Index the rRNA database: command ‘`buildtrie`’

The executable `buildtrie` indexes an rRNA database.

To see the man page for `buildtrie`,

```
> buildtrie -h

  This program builds a Burst trie on an input rRNA database file in fasta format
  and stores the material in binary files under the folder ‘automata’

     ./buildtrie --db [path to rrnas database file name {.fasta}] {OPTIONS}

  The list of OPTIONS can be left blank, the default values will be used:

     -L      length of the sliding window (the seed)
             (default: 18)

     -F      search only the forward strand

     -R      search only the reverse-complementary strand
             (default: both strands are searched)

     -h      help
```

There are eight rRNA representative databases provided in the ‘`sortmerna/rRNA_databases`’ folder. All databases were derived from the SILVA SSU and LSU databases (release 111) and the RFAM databases using the tool UCLUST. Additionally, the user can index their own database.

4.1.1 Example 1: buildtrie

```
> buildtrie --db ~/sortmerna/rRNA_databases/silva-bac-16s-database-id85.fasta

   Burst trie(s) built in:      36.7594s
   Writing Burst trie forward to silva-bac-16s-database-id85.bursttrief.dat
   Writing Burst trie reverse to silva-bac-16s-database-id85.bursttrier.dat
```

```
    Done.
```

The indexed databases (ex. `silva-bac-16s-database-id85.bursttrief.dat`) will be stored in the directory '`/some/path/to/sortmerna/automata`' (the path stored in variable `$SORTMERNADIR`, which was established in Step 1-4 of Subsection 2.2 or Subsection 2.3) and later retrieved by the command `sortmerna`, explained in the following section.

4.2 Filter reads against the indexed rRNA database: command ‘sortmerna’

The executable sortmerna filters rRNA reads against an indexed rRNA database.

To see the man page for sortmerna,

```
> sortmerna -h

  To run SortMeRNA, type in any order after ‘sortmerna’:

      --I      [illumina reads file name {fasta/fastq}]

      --454    [roche 454 reads file name {fasta/fastq}]

      -n        number of databases to use (must precede --db)

      --db     [rrnas database name(s)]
                One database,
                ex 1. -n 1 --db /path1/database1.fasta

                Multiple databases,
                ex 2. -n 2 --db /path2/database2.fasta /path3/database3.fasta
      {OPTIONS}

  The list of OPTIONS can be left blank, the default values will be used:

      --accept      [accepted reads file name]
      --other       [rejected reads file name]
                    (default: no output files are created)

      --bydbs       output the accepted reads by database
                    (default: concatenated file of reads)

      --log         [overall statistics file name]
                    (default: no statistics file created)

      --paired-in   put both paired-end reads into --accept file
      --paired-out  put both paired-end reads into --other file
                    (default: if one read is accepted and the other is not,
                    separate the reads into --accept and --other files)

      -r            ratio of the number of hits on the read / read length
                    (default Illumina: 0.25, Roche 454: 0.15)

      -F            search only the forward strand
      -R            search only the reverse-complementary strand
                    (default: both strands are searched)

      -a            number of threads to use
```

```
                    (default: 1)

    -m              (m x 4096 bytes) for loading the reads into memory
                    ex. ‘-m 4’ means 4*4096 = 16384 bytes will be allocated for the reads
                    note: maximum -m is 1020040
                    (default: m = 262144 = 1GB)

    -v              verbose
                    (default: deactivated)

    -h              help

    --version       version number
```

The command `sortmerna` takes as input a list of rRNA databases (in fasta format) and a set of Illumina or Roche 454 reads (in fasta or fastq format), and filters out the reads matching to at least one of the rRNA databases. The user has an option to output the accepted reads into a single file (default), or into multiple files sorted by the closest matching database (add the flag `--bydbs`). The indexed part of the databases created by `buildtrie` is loaded into `sortmerna` independently.

The user can adjust the amount of memory allocated for loading the reads through the command option `-m`. By default, `-m` is set to be high enough for 1GB. If the reads file is larger than 1GB, then `sortmerna` internally divides the file into partial sections of 1GB and executes one section at a time. Hence, if a user has an input file of 15GB and only 1GB of RAM to store it, the file will be processed in partial sections using `mmap` without having to physically split it prior to execution. Otherwise, the user can set `-m` high enough to store all 15GB in RAM.

4.2.1 Example 2: sortmerna on multiple databases, with output of accepted reads sorted by database

```
 > sortmerna -n 3
             --db ~/sortmerna/rRNA_databases/silva-bac-23s-database-id98.fasta
                  ~/sortmerna/rRNA_databases/silva-bac-16s-database-id85.fasta
                  ~/sortmerna/rRNA_databases/silva-euk-18s-database-id95.fasta
             --accept rrna
             --bydbs
             --454 SRR106861-filtered.fasta
             --log bilan
             -a 3
             -v

  WARNING: option ‘--other’ has been left blank, no output file for rejected reads ..

  -------------------------------------------------------
  Welcome to SortMeRNA!
  Copyright (C) 2012 Bonsai Bioinformatics Research Group
  LIFL, Université Lille 1, CNRS UMR 8022, INRIA 2012
  -------------------------------------------------------
  The size of the reads file <33862846> bytes
  will be executed in 1 partial section(s) of size
  <33862846> bytes

  [Partial section # 1]
  ---------------------
  Time to mmap reads and set up pointers:           0.2164s

  Begin analysis of: ./rRNA_databases/silva-bac-23s-database-id98.fasta

  Time to load the Burst trie:                      1.4488s
  Begin parallel traversal ...
  Time of parallel traversal of automata:           14.2974s

  Begin analysis of: ./rRNA_databases/silva-bac-16s-database-id85.fasta

  Time to load the Burst trie:                      2.2975s
  Begin parallel traversal ...
  Time of parallel traversal of automata:           17.3430s

  Begin analysis of: ./rRNA_databases/silva-euk-28s-database-id98.fasta

  Time to load the Burst trie:                      2.0302s
  Begin parallel traversal ...
  Time of parallel traversal of automata:           19.0678s

  Total number of reads found in current section:   95206


  Time to output reads to file:                     1.7558s
  Total number of reads found:                      95206
```

The option '--log bilan' will create an overall statistics file called bilan.log,

```
> cat bilan.log

 Time and Date

 Settings:
 r = 0.15
 L = 18
 reads file: ~/SRR106861-filtered.fasta

 Results:
 total reads: 105873
 non-rRNA: 10667
 rRNA: 95206
 % rRNA: 89.92%

silva-bac-23s-database-id98.fasta 64.39%
silva-bac-16s-database-id85.fasta 25.53%
silva-euk-28s-database-id98.fasta 0.009445%
```

Now, we can manually check the number of reads matched per database:

```
 > grep -c ‘>’ rrna.*

rrna.silva-bac-16s-database-id85.fasta:27026
rrna.silva-bac-23s-database-id98.fasta:68170
rrna.silva-euk-28s-database-id98.fasta:10
```

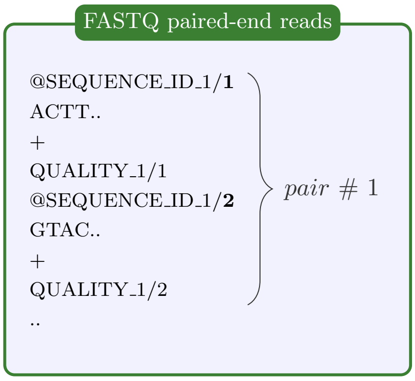
4.2.2 Example 3: sortmerna on multiple databases, with output of accepted reads directed to one single file

```
 > sortmerna -n 3
             --db ~/sortmerna/rRNA_databases/silva-bac-23s-database-id98.fasta
                  ~/sortmerna/rRNA_databases/silva-bac-16s-database-id85.fasta
                  ~/sortmerna/rRNA_databases/silva-euk-18s-database-id95.fasta
             --accept rrna
             --454 SRR106861-filtered.fasta
             --log bilan
             -a 3
             -v

  WARNING: option ‘--other’ has been left blank, no output file for rejected reads ..

  -------------------------------------------------------
  Welcome to SortMeRNA!
  Copyright (C) 2012 Bonsai Bioinformatics Research Group
  LIFL, Université Lille 1, CNRS UMR 8022, INRIA 2012
  -------------------------------------------------------

  The size of the reads file <33862846> bytes
  will be executed in 1 partial section(s) of size
  <33862846> bytes

  [Partial section # 1]
  ---------------------
  Time to mmap reads and set up pointers:           0.2184s

  Begin analysis of: ./rRNA_databases/silva-bac-23s-database-id98.fasta

  Time to load the Burst trie:                      1.4594s
  Begin parallel traversal ...
  Time of parallel traversal of automata:           14.5262s
  Begin analysis of: ./rRNA_databases/silva-bac-16s-database-id85.fasta

  Time to load the Burst trie:                      2.2632s
  Begin parallel traversal ...
  Time of parallel traversal of automata:           17.3602s

  Begin analysis of: ./rRNA_databases/silva-euk-28s-database-id98.fasta

  Time to load the Burst trie:                      1.9980s
  Begin parallel traversal ...
  Time of parallel traversal of automata:           19.4148s

  Total number of reads found in current section:   95206


  Time to output reads to file:                     0.9978s
  Total number of reads found:                      95206
```

Now, we can manually check the number of reads matched for all databases:

```
 > grep -c ‘>’ rrna.*

rrna.fasta:95206
```

4.2.3 Example 4: sortmerna on paired-end reads (1 input file - paired-end reads are interleaved)



```
@SEQUENCE_ID_1/1
ACTT..
+
QUALITY_1/1
@SEQUENCE_ID_1/2
GTAC..
+
QUALITY_1/2
..
```

Figure 2: Paired-end read format accepted by SortMeRNA

This example illustrates three cases of output for paired-end reads: default, `--paired-in` and `--paired-out`.

We use the `set6-database.fasta` found under Section 3.2 of the Supplementary file (also available online at `bioinfo.lifl.fr/RNA/sortmerna/material.php`). As for the reads, 5000 Illumina paired-end reads were simulated using MetaSim on `set6-database.fasta`. The reads were then arranged to have 2475 pairs of rRNA, and the other 25 pairs where exactly one read is rRNA and the other is not (this is possible if one of the reads covers a low complexity region on the rRNA sequence).

Remark: The statistics in the `--log` file will always give the true number of reads classified as rRNA.

Case 1: We don't care to keep the paired-end order in the output (default).

```
> sortmerna -n 1
            --db set6-database.fasta
            --I paired-end-5000-reads.fasta
            --accept rrna
            --other nonrrna
            --log bilan
            -a 1

> cat bilan.log

 Results:
 total reads:   5000
 non-rRNA:      25
 rRNA:          4975
 % rRNA:        99.5%

set6-database.fasta       99.5%

> grep -c '>' rrna.fasta nonrrna.fasta
rrna.fasta: 4975
nonrrna.fasta: 25
```

Case 2: We want to accept all pairs with at least one read that hits (`--paired-in`).

```
> sortmerna -n 1
            --db set6-database.fasta
            --I paired-end-5000-reads.fasta
            --accept rrna
            --other nonrrna
            --log bilan
            --paired-in
            -a 1

> cat bilan.log

 Results:
 total reads:   5000
 non-rRNA:      25
 rRNA:          4975
```

```
  % rRNA:        99.5%

 set6-database.fasta       99.5%

 > grep -c ‘>’ rrna.fasta nonrrna.fasta
 rrna.fasta: 5000
 nonrrna.fasta: 0
```

Case 3: We want to reject all pairs with at least one read that hits (`--paired-out`).

```
 > sortmerna -n 1
             --db set6-database.fasta
             --I paired-end-5000-reads.fasta
             --accept rrna
             --other nonrrna
             --log bilan
             --paired-out
             -a 1

 > cat bilan.log

  Results:
  total reads:   5000
  non-rRNA:      25
  rRNA:          4975
  % rRNA:        99.5%

 set6-database.fasta       99.5%

 > grep -c ‘>’ rrna.fasta nonrrna.fasta
 rrna.fasta: 4950
 nonrrna.fasta: 50
```

4.2.4 Example 5: sortmerna on forward-reverse paired-end reads (2 input files)

SortMeRNA accepts only 1 file as input for the reads. If a user has two input files, in the case for the foward and reverse paired-end reads (see Figure 3), they may use the `merge-paired-reads.sh` script found in `‘sortmerna/scripts’` folder to interleave the paired reads into the format of Figure 2.

The command for `merge-paired-reads.sh` is the following,

```
 > bash ./merge-paired-reads.sh forward-reads.fastq reverse-reads.fastq outfile.fastq
```

Now, the user may input `outfile.fastq` to SortMeRNA for analysis.

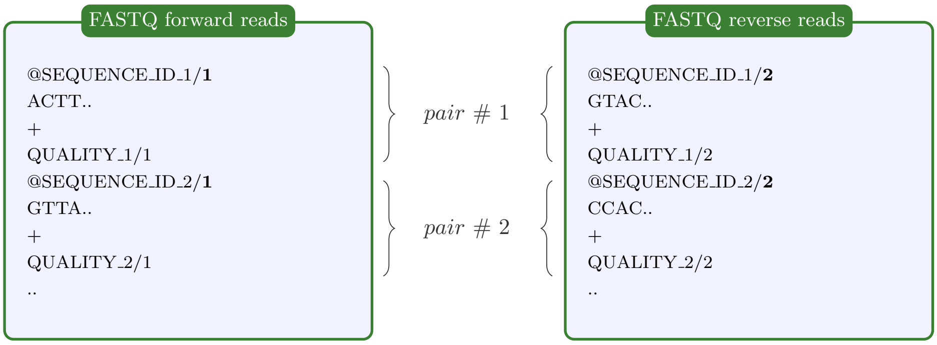


Figure 3: Forward and reverse reads in paired-end sequencing format

Similarly, for unmerging the paired reads back into two separate files, use the command,

```
> bash ./unmerge-paired-reads.sh merged-reads.fastq forward-reads.fastq reverse-reads.fastq
```

5 SortMeRNA parameters

There are two parameters in SortMeRNA which the user can moderate for performance: The length of the sliding window s (the seed), and the threshold ratio r of matching windows to the rRNA database for a read to be accepted. Both of these paramaters and their default values are discussed in detail in *Section 2: Parameter Setting* of the supplementary file. The user may adjust the threshold parameter r with the sortmerna command-line option `-r [new ratio]`. To adjust the length of the sliding window s, the user must provide the option `-L [new length (even integer)]` when indexing an rRNA database using the `buildtrie` executable.